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GNE.3030R1C5

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Goddard et al. (as amended)
Appl. No.	:	10/036,342
Filed	:	December 26, 2001
For	:	POLYPEPTIDES THAT INDUCE CELL PROLIFERATION (as amended)
Examiner	:	Kolker, Daniel E.
Group Art Unit	:	1649

DECLARATION UNDER 37 CFR §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We declare and state as follows:

1. We are the inventors of the invention claimed in the above-captioned patent application.
2. During the time period in which we participated in the events and activities described herein, we were employed by Genentech, Inc., the assignee of the above-captioned application.
3. All of the events and activities described herein were performed by us personally, or by others at our direction as part of our duties as employees of Genentech, Inc.
4. The invention claimed in the above-captioned patent application was conceived and reduced to practice in the United States prior to November 10, 1999 as described below.
5. Prior to November 10, 1999, we conceived of the invention claimed in the above-captioned patent application. This is demonstrated by the attached sequence printout (Exhibit A), which was generated prior to November 10, 1999, and which shows the complete sequence of the nucleic acid having the sequence of SEQ ID NO: 56. The attached printout also shows the complete sequence of the polypeptide which has the sequence of SEQ ID NO: 57. As evidenced by the sequence printout, we were in possession of the complete nucleic acid and amino acid sequences prior to November 10, 1999.
6. The date deleted from Exhibit A is prior to November 10, 1999. This date was redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, April 28, 2005.
7. After these initial experiments, we diligently reduced the claimed subject matter to practice by working to express and purify the encoded polypeptide and to run it systematically

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through many assays. The cDNA was deposited with the American Type Culture Collection (ATCC) on April 20, 1999 and assigned ATCC no. 203948. The protein of interest was assigned a "protein inventory number" (e.g., PIN1205-1), and this protein is a polypeptide having the sequence of SEQ ID NO:57, and is encoded by SEQ ID NO: 56.

8. Exhibit B shows that the protein lot designated PIN1205-1 was delivered to James Pan on a date prior to November 10, 1999 in order to perform assay ASY92, called "Mouse Mesangial Cell proliferation Assay." Also, as shown in Exhibit B, the assay was completed on a date prior to November 10, 1999. Exhibit B also shows that the tested polypeptides tested positive ("All Positives"), thereby confirming the ability of the encoded polypeptide to induce mesangial cell proliferation. Thus, actual reduction to practice occurred on a date prior to November 10, 1999.

9. The dates deleted from Exhibit B all are prior to November 10, 1999. These dates were redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, April 28, 2005.

10. After reducing the invention to practice, we worked with the Genentech, Inc. patent department to prepare a non-provisional patent application, which included the sequences of SEQ ID NO:56 and SEQ ID NO:57, as well as the data showing the ability to induce mesangial cell proliferation. That application was filed on March 1, 2000 as PCT/US00/05601.

11. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: 
Audrey Goddard

Date: 19 Oct 05

By: _____
Paul J. Godowski

Date: _____

By: _____
Austin L. Gurney

Date: _____

By: _____
James Pan

Date: _____

By: _____
Colin K. Watanabe

Date: _____

By: _____
William I. Wood

Date: _____

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By: _____

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Colin K. Watanabe

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William I. Wood

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Date: 10/18/05

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By: _____
Audrey Goddard

Date: _____

By: _____
Paul J. Godowski

Date: _____

By: _____
Austin L. Gurney

Date: _____

By: _____
James Pan

Date: Oct 24/05

By: _____
Colin K. Watanabe

Date: _____

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William I. Wood

Date: _____

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James Pan

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By: Colin K. Watanabe
Colin K. Watanabe

Date: Oct 20, 2005

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James Pan

Date: _____

By: _____
Colin K. Watanabe

Date: _____

By: William I. Wood
William I. Wood

Date: 10/16/05

>Thursday, April 28, 2005
>DNA92234 [Full]
>887 Sites [All Sites] [DNA92234], sheldens
> Lib309

>Sequence confirmed by phredphrap

thAI
nlaIII snuBII
spHI fnuDII/mvuI mnlI
nspHI bstUI tail taqI
tail nspI bsh1236I tsp509I [M-ecori-]
maeII/hpyCH4IV bsiWI/splI
aluI hinIII/acyI cac8I bsaAI ecorI tliI
sapI aharII/bsaBII mluI rsal hpy188I acII hpy188I acII
maeIII mbolI atII cac8I afillI maeII/hpyCH4IV paeR7I hpy188I acII
hphI sfcI earI/ksp632I hpy99I hpyCH4V csp6I aluI apol avail [M.taqI-] mnlI fnu4HI/bsoFI hpy188I
1 TAGGTGACAC TATAGAAGAG CTATGACGTC GCTATGCAACG GATACGTAGC TOGGAATTC GCTCGAGGAA TGATAACCTC CGAAGCCCTC TTGTTCGCA
ATCCCACTCTG ATATCTCTC GATACTGCAAG CGTACGTGGG GATGCCATTCG AGCCTTAAGC CGAGCTCCCT ACTTATGGAG GCTTCGGCA AACAAAGGCT

^insert starts here

scrFI [M. hpaII-]
 nciI
 mspI
 hpaII
 dsav
 bpuII bssKI
 bbsI bsII bsmFI tail
 mnII mbII bsauI
 aluI
 101 CTTGTGAAATA GCTCCCTCAT ACCAGCCCTCG TCTTCCCTTC GGGGGACAAAC GTGGGTCAAG GCACAGAGAG ATATTTAATG TCACCCCTCTT GGGGCTTCA
 CTACACTTAT CGAGGTATA TGGTGGAGC AGAAGGAAGG CCCCTCTTG CACCCAGTCC CGTGTCTCTC TATAAATTAC AGTGGGAGAA CCCCGAAAGT

bsp1286
 bmyI
 maeII/hpych4IV
 bsp45I
 tru9I hphI
 mseI maeIII nla

sau3AI
 mbol/ndelII [dam-]
 dpnII [dam-]
 dpnI [dam-]
 alwI [dam-]

nlaIV
 bstXI/xbaII
 hgaI

mnII
 rnaI
 maeI
 bsmFI mnII
 201 TGGGACTCCC TCTGCCACAT TTTTGAGG TGGGAAAGT TGGTGGGG TTCAAGTC CAGCTTAATG GATCCAAAC TCGCTGGGTCC
 ACCCTGAGGG AGACGGTGTAA AACCCCTTC ACGATCTCCG AAGCTTGA GTCGGATTAC CTAGGGTTG AGCCCTCTTA CCGACGAGG

M D P K L G R . N A A S
 ^MET

scrFI [dam-] mnlI
 pspGI bpuI/gsuI [dam-]
 mvaI sau3AI scrFI [dam-]
 ecoRII [dam-] pspGI
 dsaV [dam-] mboI/ndeII [dam-]
 bstNI dpnII [dam-]
 bsp1286 bstYI/xbaII
 bmyI bssKII [dam-] mboII
 hpy188I apyI [dam+] dpnI [dam+]
 eco57I bsaJI bglII
 mwoI banII bpuI/gsuI [dam-]
 801 GGCCTTCAGA GCCCTGGAGC AAGATTTTCG TGTGAAATTC AAATTGCTCA TTAGGGCAT GGAAAGGGCT GGCTCTCTTG CCCTGGAGGA ACTTGCGAA
 GGGAAAGTCG CGGGACCTCG TTCTAGGG ACACATAG TTTAAGTAGT AACTCCCTAA CCTTCTCGGA CCGAGACAC GGGACCTCTT TGAACACCTT
 179 A F R A L E Q D L P V N I K F I I E G M E E A G S V A L E S L V E

scrFI [dcm-]
 pspGI
 mvaI
 ecoRII [dcm-]
 dsAV [dcm-]
 bstNI
 bssKI [dcm-]
 sau96I [dcm-]
 nlary
 accI
 scrFI [dcm-]
 pspGI pleI
 mvaI mlyI
 ecoRII [dcm-]
 dsAV [dcm-]
 bstNI hinFI
 bssKI [dcm-]
 apyI [dcm+]
 mboII
 1101 TCTTCCTGGT AGCCCTGGAG ACTCGTCTGG TCATATCCTG GTCCCTGGA TCTATGATGA AGTGGTCCCT CTTACAGAG AGAATATAAA TACATACAA
 AGAAGAGCCA TCGGACCCATC TGAGCAGGCC AGTATAGGAC CAGGGACCT AGATACACT TCACCAAGGA GAATGTCTC TCTTTATT ATGATGTT
 279 L L G S I V D S S G H I L V P G I Y D E V V P L T E E I N T Y K

GSeqEdit, DNA92234 [Full], page 11

sau3AI
 mboI/ndelII [dam-]
 dpnII [dam-]
 fokI dpnI [dam+]
 bstF5I
 scrFI [M.hpaII-]
 nciII
 alwI [dam-]
 msplI
 nlaIV
 hpaII bstYI/xbaII
 dsav bamHI
 bssKI alwI [dam-]
 bssKI ATCCACCATT CCATTGCCA AAATGTTCCA GGAGATGTC CACAAGAGCG TGGTCTTAAT TCCCGTGGGA GCTGTGTGAG ATGGAGAAC
 1601 TCGGGATGG TAGCTTACCGTTAGGTCTCTAGGT CTCAGGGT ACCACATTA AGGGGACCCCT CGACAACTAC TACCTCTGT
 AGGCCCTTAC TAGCTGGAA GGTTAACGGT TTACAGGT CTCAGGGT CTCAGGGT ACCACATTA AGGGGACCCCT CGACAACTAC TACCTCTGT
 446 R D G S T I P I A K M F Q E I V H K S V V I I P I G A V D D G E H
 tru9I
 tseI
 fnu4HI/bsoFI
 mlaIV
 msp509I bbVI
 msp509I ddeI
 1701 TTGGCAGAT GAGAAATCA ACAGGGAA CTACATAGAG GCAACCAAT TATTGCTGC CTTTTCTTA GAGATGGCCC AGCTCCATTAA ATCAGAGA
 AGGGCTTAA CTCTTTAGT TCTCCACCTT GATGTATCTC CCTGGTTA ATAAAGACG GAAAGAAAT CTCTACCGGG TCGAGGTAAAT TAGTGTCTT
 479 S Q N E K I N R W N Y I E G T K I P A A F F L E M A O L H O

scrFI [dam-]
 pspGI
 mvaI
 ecoRII [dam-]
 dsaV [dam-]
 bstNI
 bssKI [dam-]
 apyI [dam+]
 bsII
 tfII
 hpyCH4V
 bsAU
 hinFI
 smLI
 bbVI
 hpyCH4V
 aluI
 mnuI
 2001 CCTTCCTCAA GTCATAGCTG CTTCAGCAA CTGATTCC CCAAGTCTG TGCAATAGCC CCAGGATTGG ATTCCCTCCA ACCCTTACG ATATCTCAA
 GCAAGGAGTT CAGTATGAC GAACTAAAGG GTCAGGGAC ACGTATCCG GTTCAGGAC AGTCATCCG GTTCAGGAC TACGAGGAC ATTACGAGT TGTAGGCT TATAGAGGT

 sau96I
 top45I
 avaII
 bssSI
 hgiAI/aspHI
 ppMI
 eco0109I/draI hpy188III
 sau3AI
 mboI/hdeII [dam-]
 bsp1286
 xbaI
 fokI
 dpnII [dam-]
 msPI
 hpaII
 smLI
 bsiHKAI
 ppMI
 bfaI
 mnuI
 bmyI
 maeIII
 bstF5I
 dpnII [dam+]

 top509I
 hpyCH4V
 2101 CCTTCCTCAA TGATGGCAT AATCACCTCG GTTGCTTC TAGTCTCCTCA AGTGCTGTG AGACATAATC ATTCCATCCA ARGATCGCCT TTGCTTACCC
 GAAACGTTA ACTAACCGTA TTAGTGGGC CAAAGAAG ATCCAGGAGT TGCTGTTAG TGTGTTAGG TACTAGGGAA AACGAAATGG

 tru9I
 mseI
 bsmAI
 asel/asnI/vspI
 bsAI
 tsPRI
 2201 ACTCTTCTCT TTTATCTAT TAATRAAAT GTGCTCTCC ACCACTGNT CCCAAAAA AAAAAGAAA AAAAAGAAA AAAAAGAAA
 TGAGAAGCA AAATAGAATA ATTATTTA CAACAGAGG TTGTGACNGA GGTTTTTTT TTGTGTTTTT TTGTGTTTTT TTGTGTTTTT TTGTGTTTTT

> length: 2425

aatII (GACGTC) :	25	1295 2374
acc65I (GCTTACC) :		727 1117 2348
accI (GTTKAC) :		
accIII (TCCGGA) :	2366	
aciI (CCGC) :		86 332 355 511 1420 1672 2326 2330
acyI (GRCGYC) :	25	
aiIIII (ACRYGT) :	37	
ageI (ACGGT) :	23771	
ahalII (GRCGYC) :	25	
ahalIII (TTAAA) :		1914
aluI (AGCT) :		19 48 110 485 569 1006 1680 1781 2016 2343 2392 2419
alw26I (CAGNNNNCTG) :	418	523 565
alwI (GGATCCNNNN) :	270	271 628 785 959 1319 1599 1609 1610 1817 1936
alwNI (CAGNNNNCTG) :	418	523 565
apal (GGGCCC) :	533	
apoI (RATTI) :		54 409 841 1249 1381 1879
apyI (CCWGG) :		528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
aseI (ATTAAT) :		1787 2219 2360
asnI (ATTAAT) :		1787 2219 2360
asp700 (GAANNNTTC) :		375 1159 1379 1469 2358
asp718 (GGTACCC) :	1295	2374
aspHI (GNGGMC) :		484 2152 2342
asPI (GACNNNGTC) :	451	
avaI (CYCERG) :		62 280 995 2353
avaII (GTTGCC) :		559 705 909 1140 1985 2143 2369
baII (TGGCCA) :		437
bamHI (GGATCC) :		270 1609
banI (GGTCCC) :		640 1295 2374

GSeqEdit, DNA92234 [Full], page 16

banII (GGGCGC) : 484 533 809 2342
bbsI (GAAGACANNNNN) : 130 379 587
bbVI (GCAGGC) : 292 312 315 318 321 508 519 522 567 570 672 1235 1552 1756 2017 2024
bceAI (ACGGC~~NNNNNNNNNNNN~~) : 502 656
bfaI (CTAG) : 243 1210 1216 1396 1504 1805 1849 1889 2140 2337
bgII (GCCNNNNNGGG) : 535
bgIII (AGATCT) : 822
bmyI (GDGCHC) : 159 484 533 809 2152 2342
bpMI (CTGGAG) : 96 258 325 814 883 1290
bpUAI (GAAAGC~~NNNNNN~~) : 130 379 587
bsaAI (YACGTR) : 42
bsaII (GRGGYC) : 25
bsaI (GGTCTC~~NNNNNN~~) : 1034 2234
bsaJII (CCNNGG) : 139 359 503 528 545 684 812 881 995 996 1143 1516 2060 2353
bsaWI (WCCGGG) : 1226 2127 2366 2371
bsERI (GAGGAGGNNNNNNNN) : 342 749 1270
bsgI (GTGCAG) : 415 670 1994
bshI236I (CGCGCG) : 38 331 1329
bsiEI (CGRYCG) : 755 2327
bsiHKAI (GRGGC~~RC~~) : 484 2152 2342
bsiWI (CGTACG) : 40
bsI1 (CCNNNNNNNGG) : 135 184 274 275 354 396 614 631 771 1847 1848 2060
bsmAI (GTC~~TC~~) : 1034 2235
bsmAI (GTCTC) : 1034 2235
bsmFI (GGGAC~~NNNNNNNNNNNN~~) : 143 202 297 1141 1399 1986
bsOFl (GNGGC) : 85 292 312 315 318 321 332 508 519 522 567 570 672 1235 1552 1756
bsp120I (GGGCC) : 2017 2024 2326 2329 533
bsp1286 (GDGCHC) : 159 484 533 809 2152 2342
bspCNI (CTCAG~~NNNNNNNN~~) : 563 1050

bsPEI (TCCGGA) : 2366
bsPHI (TCATGA) : 1074
bsPMI (ACCTGC) : 2377
bsPMII (TCCGGA) : 2366
bsREFI (RCGGY) : 2371
bsRI (ACTGGN) : 384 618 1542
bsSKI (CCNGG) : 139 360 528 609 684 813 882 995 996 1038 1113 1137 1144 1239 1342
1363 1602 1638 2061 2353 2354.
bsSSI (CTCGTG) : 2155
bst4CI (ACNCT) : 643 1354 1573
bstAPI (GCANNNNNTGC) : 641
bstDSI (CCRYGG) : 503 1516
bstF5I (GGAATG) : 405 606 857 1068 1203 1605 1844 1857 2175
bstN1 (CCYGG) : 528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
bstU1 (CGCG) : 38 331 1329
bstX1 (CCANNNNNTGG) : 260 1478
bstY1 (RGATCY) : 270 822 1609
btGI (CCRYGG) : 503 1516
btRI (CACCTC) : 667
btSI (GCAGTGAN) : 1992
cacB1 (GCGNGC) : 31 35 303 675 868 975 2020 2381
cfol (GCCG) : 330 364 525 800 1328
cfri10I (RCGGY) : 2371
cfri11YGGCR) : 437 500 611 657 1365 2327
cpo1 (GGGCGG) : 2368
csp6I (GTAC) : 41 387 1296 1897 2375 2387
cspI (GGGCCG) : 2368
ddel (CTNAG) : 563 1050 1265 1767
dpnI (GATC) : 271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
2183

hincII (GTYRAC) :	2348
hindII (GTYRAC) :	2348
hinfI (GANTC) :	204 451 585 914 1120 1148 1275 1500 1829 2070 2407
hinII (GRCGYC) :	25
hpII (CCGG) :	139 361 684 996 1227 1239 1602 2128 2354 2367 2372
hphI (GGTGA) :	3 181 346 1023 1434 1832
hpy188I (TCNGA) :	51 79 252 476 491 582 806 946 1568 1809 1814
hpy188III (TCNGA) :	97 281 402 443 1051 1074 1209 1289 1446 1873 1933 2156 2366
hpy99I (CGWCG) :	27 2347
hpyCH4III (ACGTC) :	643 1354 1573
hpyCH4IV (ACGT) :	26 43 149 668
hpyCH4V (TGCAC) :	34 416 521 671 1030 1283 1524 1995 2023 2051 2104 2380
kpnl (GGTAC) :	1295 2374
ksp632I (CTCTTCNNNN) :	15 487 862 1100 1177
maeI (CTAG) :	243 1210 1216 1396 1504 1805 1849 1889 2140 2337
maeII (ACGT) :	26 43 149 668
maeIII (GTAC) :	4 180 1435 2158
mboI (GATC) :	271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
mboII (GAAGA) :	2183
mcrI (CGRYCG) :	15 131 380 488 588 825 862 917 1101 1177 1219 1450
mfeI (CAATTG) :	755 2327
mluI (ACGCC) :	1622
mluI (CGCTT) :	37
mlyI (GAGTCNNNN) :	204 451 585 1120 1500 2407
mnII (CCTC) :	65 77 126 185 209 227 246 344 350 396 469 545 562 598 724 749 853
mroI (TCCCGA) :	865 886 1021 1168 1180 1287 1293 1324 1402 1738 1835 2005 2146
mscI (TGGCCA) :	2366
mscI (TGGCCA) :	437
mscI (TTAA) :	175 1788 1915 1981 2220 2361
mscI (TAA) :	400 1405 1407

mspII (CGGCKG) : 568 1672
mspI (CCGG) : 139 361 684 996 1227 1239 1602 2128 2354 2367 2372
mnuI (CAATTG) : 1622
mvaI (CCWGG) : 528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
mvrI (CGCG) : 38 331 1329
mwol (GCNNNNNNNGC) : 303 312 315 321 357 502 535 641 650 793 802 1555 1665
ncII (CCSGG) : 139 360 684 995 996 1239 1602 2353 2354
ndelII (GATC) : 271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
nlalII (CATG) : 2183
nlalIV (GCAAGCC) : 32 199 336 555 1014 1075 1315 1407 1497
notI (GGGGCCGC) : 270 532 533 558 640 705 991 1054 1140 1164 1295 1609 1741 1985 2374
nspBII (CGGCKG) : 2326
nspHI (RCATGCT) : 568 1672
nspI (RCATG) : 31 335
nspI (RCATG) : 31 335
paeR7I (CTCGAG) : 62
palI (GGCC) : 438 501 534 543 612 658 769 1366 1776 2328
pf1FI (GACNNNGTC) : 451
pleI (GAGTCNNNN) : 204 451 585 1120 1500 2407
ppuMI (RCGWCCT) : 558 1984 2142
pshAI (GACNNNNGTC) : 553
pspAI (CCCGGG) : 995 2353
pspGI (CCWGG) : 528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
pspOMI (GGGCC) : 533
pstI (CTGCA) : 520 2379
pyrII (CAGCTG) : 568
rcal (TCATGA) : 1074
rmaI (CTAG) : 243 1210 1216 1396 1504 1805 1849 1889 2140 2337
rsal (GTAC) : 41 387 1296 1897 2375 2387
rsrII (CGGRCGG) : 2368

sacI (GAGCTC) : 484 2342
salI (GTCGAC) : 2348
sapI (GCTCTTCNNNN) : 15 486 1099
sau3A1 (GATC) : 271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
2183
sau96I (GNNCC) : 533 534 559 705 769 909 1140 1776 1985 2143 2369
sbfI (CCTGCAGG) : 2378
scrFI (CCNGG) : 139 360 528 609 684 813 882 995 996 1038 1113 1137 1144 1239 1342
1363 1602 1638 2061 2353 2354
sf3A1 (GCATC) : 1067
sfCI (CTRYAG) : 10 520 2379 2400
sfII (GGCCANTNNNGGG) : 534
smal (CCCGG) : 995 2353
smLI (CTYRAG) : 62 2006 2147
snBI (TACGTA) : 42
speI (ACTAGT) : 23336
spHI (GCATGC) : 31
spII (CGTACG) : 40
spIII (CGTACG) : 2378
sse8387I (CCCTGGAGG) : 1528 1949
ssPI (AATATT) : 484 2342
sstI (GAGCTC) : 26 43 149 668
taII (ACGT) : 63 443 1259 1322 2349
taqI (TCGA) : 914 1148 1275 1829 2070
tfII (GAWTC) : 38 331 1329
thaI (CGCG) : 62
tliI (CTCGAG) : 175 1788 1915 1981 2220 2361
tru9I (TAA) : 292 312 315 318 321 508 519 522 567 570 672 1235 1552 1756 2017 2024
tseI (GCAGG) : 4 180 1435 2158
tsp45I (GTSAC) : 55 410 842 942 1250 1382 1623 1668 1748 1880 2107 2359 2363
tsp509I (AATT) : GSeqEdit, DNA92234 [Full], page 22

tertI (NNCAAGTGN) :	1574	1821	1992	2243
tth111I (GACNNNGTC) :	451			
vspI (ATTAAAT) :	1787	2219	2360	
xbai (TCTAGA) :	1209			
xhol (CTCGAG) :	62			
xhoII (RGATCT) :	270	822	1609	
xmaI (CCCGGG) :	995	2353		
xmaII (CGGGCG) :	2327			
xmaIII (GATNNNNTC) :	375	1159	1379	1469
				2358

Sample Name	ASY192
Protocol Name	Mouse Mesangial Cell proliferation Assay
Protocol Alias	ASY192
Protocol Status	Retired
Protocol Class	Primary
Protocol Format	96 Well
Protocol Type	Cell
Protocol Requirements	
Protocol Assay Volume	0.1 ml
Protocol Fold Out Into Well	10 Fold
Protocol Replicates	3
Protocol Dilutions	2
Protocol Volume Requested	0.025ml/well/300ul
Protocol Species	Mouse
Protocol Purpose	Screen SP60 proteins which can stimulate Mesangial Cell Proliferation
Protocol Protocol	On day 1: 10 ⁵ cells measured/plate in Medium/A51 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium/0.05% fetal bovine serum/5% CO ₂ supplemented with 14-nM heparin and 10% growth supplement. On day 2: SP60 Proteins are diluted at 1:2 in Medium/A51 in serum-free Medium and added to the cells. On day 4: After 48 hours incubation- each well of the plate was added 20 μ l of the Cell Titer 96 aqueous one solution reagent [Promega] and colorimetric reaction was allowed for 2 hours. The absorbance [OD] is measured at 490 nm.
Protocol Matrix	Promega kit for the assay
Protocol Result Calculation	replicated average
Protocol Result Interpretation	Any PI% that gives an absorbance reading which is 15% above the media control is considered a hit.
Protocol Result CutOff	> 15 %
Protocol In Vitro In Vitro	
Comments	
Status	Retired
Date Entered	
Date Cancelled	
Department	Endocrinology
Submitter	Jamila (jamila123)
Notesbook	0
Assignees	

ASY192

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